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Deposition of Aerially Applied Tebufenozide (RH5992) on Balsam Fir (*Abies balsamea*) and Its Control of Spruce Budworm (*Choristoneura fumiferana* [Clem.])

Beresford L. Cadogan,* Dean Thompson, Arthur Retnakaran, Roger D. Scharbach, Arthur Robinson & Bozena Staznik

Natural Resources Canada, Canadian Forest Service—GLFC, 1219 Queen St. E, Sault Ste. Marie, Ontario, P6A 5M7, Canada

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Abstract: A field trial was conducted in 1994 to determine the foliar deposit of tebufenozide (RH5992), applied aerially, and its efficacy against spruce budworm, *Choristoneura fumiferana* (Clem.). A commercial 240 g litre^{-1} formulation of the insecticide (Mimic 240LV) was mixed with water, dyed with a tracer dye (Rhodamine WT) and sprayed with a light fixed-wing aircraft. Six application strategies were tested. Five used 70 g AI ha^{-1} in a spray volume of 1 or 2 litre^{-1} ha^{-1} with single or double applications; the sixth was an unsprayed control.

Results show that the spectra of the spray applications were, with one exception, fairly uniform. Volume and number median diameters ranged from 100 to 130 μm and 27 to 72 μm, respectively. Mean number of drops cm^{-2} on Kromekote cards were $<2{\cdot}0$ for strategies where either 1 or 2 litre ha^{-1} were sprayed. Nevertheless no one strategy produced droplet densities that were significantly different ($P < 0{\cdot}05$) from the other strategies. Tebufenozide recovered from foliage averaged 2·5 to 5·9 μg g foliage^{-1} when 1 litre ha^{-1} was sprayed and 5·8 to 6·8 μg g foliage^{-1} after 2 litre ha^{-1} were sprayed. When a single application was the strategy used, the mean number of droplets cm^{-2} and μg tebufenozide g foliage^{-1} ranged from 1·2 to 1·4 and 2·5 to 5·9, respectively. With double applications, the same response parameters ranged from 0·3 to 1·9 and 2·5 to 6·8, respectively. Budworm population reductions (%) and the number of larvae that survived tebufenozide treatments were significantly different ($P < 0{\cdot}05$) from the controls. After strategies that used 1 litre spray ha^{-1}, mean percentage population reductions ranged from 61·4 to 93·6 whereas populations were reduced by 85·6 to 98·3% when 2 litre ha^{-1} were sprayed. After double applications the mean percentage population reductions ranged from 93·6 to 98·3, but single application strategies resulted in mean reductions of 61 to 86%. Mean population reductions in the controls were 61%. The mean number of larvae per branch that survived spray strategies of 1 litre ha^{-1} ranged from 1·3 to 7·4, and from 0·4 to 1·3 when 2 litre ha^{-1} was the spray volume. In the controls an average of 10·2 larvae survived. With one exception, mean percentage defoliation in the treated areas was also significantly less ($P < 0{\cdot}05$) than that in the control. Mean defoliation in trees sprayed at 1 litre spray ha^{-1} ranged from 40 to 62·8% whereas those treated at 2 litre ha^{-1} had mean defoliation levels from 31·5 to 62·8%. In contrast, average defoliation in the controls was 92·1%. When a single application was the spray strategy, mean defoliation ranged from 31·5 to 62·8%. These data imply that a double application of tebufenozide at 70 g in 2 litre ha^{-1} was the most efficacious strategy. However, analyses of the data also show that the primary influence on deposits and defoliation was interactions between number of applications and spray. Nevertheless the two independent variables acted without significant interactions when influencing percentage reductions of spruce budworm populations. © 1998 SCI

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Key words: tebufenozide; RH5992; spruce budworm; *Choristoneura fumiferana*; aerial application

* To whom correspondence should be addressed.
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1 INTRODUCTION

Spruce budworm, *Choristoneura fumiferana* (Clem.), is the most destructive insect defoliator of the spruces, *Picea* sp., and balsam fir, *Abies balsamea* (L.) Mill., in North American forests.[1] Therefore, this insect has been the focus of a significant proportion of Canadian forest pest management research and operational strategies. For example, in 1993, approximately 78% of insecticides used operationally in forests were devoted to suppressing spruce budworm populations (unpublished report of the 21st Annual Forest Pest Control Forum, Ottawa, 1993).

Balsam fir occurs from the Rocky Mountains in the west to the Atlantic Ocean[1,2] and is one of the most abundant coniferous species in the Acadian, Great Lakes-St. Lawrence and Eastern-Central Regions of the Canadian boreal forest.[2] Balsam fir is only one of the preferred hosts of *C. fumiferana*, the others being white, black and red spruce (*P. glauca* [Moench] Voss, *P. mariana* Mill. and *P. rubens* Sarg., respectively), but it is the most susceptible of the four to budworm damage. Mortality of mature balsam fir stands usually occurs after three years of continual heavy budworm feeding,[1] whereas spruces can survive, albeit considerably weakened, even after six to eight years of defoliation.

Traditionally, epidemic outbreaks of spruce budworm have been treated with wide-spectrum chemical insecticides such as fenitrothion and aminocarb. Since the 1980s there have been strong appeals to replace such insecticides with products that are insect-specific and environmentally benign. As a consequence, biological insecticides such as *Bacillus thuringiensis* Berl. and nuclear polyhedral viruses are being used with increasing frequency to suppress forest insect pests. Insect growth regulators (IGR), although not true biopesticides, are more environmentally acceptable than wide-spectrum pesticides and have been considered as potential replacements for the latter.[3] Tebufenozide (RH5992; *N'-tert*-butyl-*N'*-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide; Fig. 1) is a novel synthetic moult-accelerating compound that belongs to a class of insect growth regulators that are non-steroidal ecdysone agonists in lepidoptera.[4] This class of compound mimics the action of 20-hydroxy ecdysone[5] and induces a premature, incomplete moult that inhibits larval feeding and ultimately leads to the insect's death.[4,6,7] Laboratory studies have demonstrated the effects of tebufenozide against corn earworm, *Helicoverpa zea* (Boddie) and fall armyworm, *Spodoptera frugiperda* (J. E. Smith).[8] The compound was also found to be active against five species of Lepidoptera, but was inactive against Coleoptera, Orthoptera and Heteroptera.[9] Tebufenozide was also found to be highly toxic to gypsy moth, *Lymantria dispar* L., hemlock looper, *Lamdina fiscellaria fiscellaria* Gueneé, and spruce budworm, *Choristoneura fumiferana* (Clem.) (B. V. Helson, 1993, pers. commun.) but exhibited low toxicity to mammals and other wildlife species of environmental concern.[10]

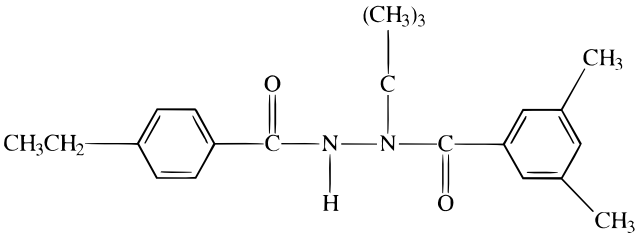

Fig. 1. Chemical structure of tebufenozide (RH5992).

This research was conducted to determine the foliar deposit and efficacy of tebufenozide applied aerially as different treatment strategies against spruce budworm and to examine relationships between its deposits on artificial substrates and those on current year balsam fir foliage.

2 MATERIAL AND METHODS

2.1 Study site and experimental design

A field trial was conducted in 1994 in a naturally regenerated boreal spruce/fir deciduous forest approximately 25 km north-east of the town of Longlac, bounded by 49°51·5′ to 49°58′ N latitude and 86°26·5′ to 86°33·5′ W longitude. Tree heights ranged from 11·9 to 13·9 m ($\bar{x} = 13$ m; $n > 120$ trees), with diameters at breast height (DBH) ranging from 12·4 to 20·1 cm ($\bar{x} = 16{\cdot}3$ cm; $n > 120$ trees). Four 50- to 70-ha blocks (1·0 to 1·7 km × 0·5 km) were prepared within the forest, with one block allocated for an experimental spray.[11,12] Five plots (approx. 5–10 ha in area) outlined as transects were prepared in each block as pseudoreplicates.[13] In addition, eight 10-ha plots were randomly selected as checks in unsprayed areas of the forest. Ten to twelve sample trees (balsam fir) were randomly selected in each plot. In the plots designated for experimental sprays, any branches of deciduous trees that might have obstructed spray from depositing on a neighbouring sample tree were removed. In addition, a 300-ha block was designated for a semi-operational spray treatment, also referred to as pilot test.[11,12] Five plots (each approximately 20 ha in area) were also randomly selected in this block as pseudoreplicates, each with 10–12 sample trees. However, as is customary in pilot tests, no special preparation of sample trees was done in these plots. As a consequence, we observed that 40–60% of the sample

trees in these semi-operational plots were shaded to some degree by overhanging deciduous trees.

2.2 Spray applications

The tebufenozide was applied as a commercial 240 g litre^{-1} formulation ('Mimic' 240 LV; Rohm & Haas Co. Inc., Springhouse, PA, USA). The product was mixed with water and a tracer dye (Rhodamine WT, 1 g litre^{-1}, A. S. Patterson, Willowdale, Ontario, Canada) was added to the spray mix to accommodate visual deposit analyses. Two Cessna 188 Ag-Truck aircraft fitted with identical spray equipment (four Micronair AU 4000 rotary atomizers), navigational aids (differential global positional systems [DGPS] Picodas Group Inc., Richmond Hill, Ont.) and data loggers (CR10, Campbell Scientific [Canada] Corp. Edmonton, Alberta, Canada)[14] were used to apply the sprays. The aircraft flew at approximately 176 km h^{-1} using 40-m flight lane separations 15 to 20 m above the forest canopy with the atomizers revolving at 4200 to 5440 rev min^{-1}.

Prescribed treatments were 70 g AI ha^{-1} in 1 and 2 litre spray ha^{-1} as either single or double applications (Table 1). When the blocks were sprayed, balsam fir buds had flushed and flared, which corresponds to a class #5 level of phenological development.[15] Larval development of spruce budworm was primarily 4th and 5th instar.

An 18-m metal tower with a set of weather monitoring equipment (a windvane-anemometer, two temperature-relative humidity probes, a rain gauge and a datalogger) was erected as a weather station within the research area to monitor weather conditions (relative humidity, temperature and air stability, rainfall, wind speed and direction). Detailed monitoring was done from 1 h before until 1 h after each spray application to allow, if possible, choice of the best spray conditions under which to apply the sprays. Conditions that existed at the spray release height during spray applications were monitored within the spray aircraft.[16] In addition, daily weather conditions (rainfall and temperature) were recorded over the course of the field study.

2.3 Assessing spray deposit

A Kromekote card (10 × 10 cm) cut into 1-cm wide strips (to allow air to flow through, rather than around the card) was installed, as described in a previous report,[17] to record spray deposit in the mid-crown of six randomly selected sample trees in each spray plot. The cards were removed approximately 1 h after the spray. Approximately 40 min after each spray, two 30-cm branch tips were taken from the mid-crown of each of five random sample trees in each plot. New shoots, i.e. current year's growth ($n \geq 159$ shoots) were clipped with scissors from the branches without disturbing the deposit, placed in wide-mouth amber bottles to minimize degradation of the tebufenozide by ultraviolet light, and frozen (−30°C) until they could be analysed with high-performance liquid chromatography (HPLC) to quantify the mass of tebufenozide deposited on current year foliage. Samples (500 ml) of the tank mixes were also analysed (HPLC) to verify tank mix concentrations.

2.4 Extraction and clean-up

Foliar samples were defrosted, thoroughly hand-mixed and extraneous materials (particularly spruce budworm

TABLE 1
Treatment and Application Details for Tebufenozide Sprays

		Application					*Tank mix concentration (g AI litre^{-1})*	
			Volume litre ha^{-1}		*Flow rate (litre min^{-1})*			
Treatment[a]	*Reference*	*Date (time)*	*Designated*	*Observed*[b]	*Calculated*[c]	*Observed*[b]	*Expected*	*Observed*[d]
70 × 1 × 2	E1	1st 14 June (06:42)	1·0	1·21	12·0	14·0	70	58
		2nd 19 June (06:00)		1·38		16·6		59
70 × 2 × 2	E2	1st 14 June (05:55)	2·0	1·97	24·0	24·9	35	32
		2nd 19 June (05:42)		1·83		23·4		36
70 × 2 × 1	E3	14 June (06:20)	2·0	1·97	24·0	24·9	35	32
70 × 1 × 1	E4	14 June (06:15)	1·0	1·24	12·0	14·8	70	58
70 × 2 × 2	S5	1st 14 June (07:53)	2·0	2·47	24·0	28·5	35	39
		2nd 18 June (21:22)		2·10		25·9		32

[a] g AI ha^{-1} × litre ha^{-1} × no. of applications; E = Experimental, S = Semi-operational.
[b] Recorded with the aircraft's data logger.
[c] Using 40-m swath intervals and airspeed of 176 km h^{-1}.
[d] Determined by HPLC.

larvae) were removed. A subsample (5 g), was then extracted with acidic methanol following previously published methods.[18] After rotary evaporation and transfer to hexane, extracts (1 ml extract, equivalent to 1 g fresh mass of foliage) were cleaned up *via* adsorption chromatography on Florisil micro-columns, and eluted with acetone + hexane (10 + 90 by volume; 15 ml). The eluate was evaporated to dryness under nitrogen, and redissolved in methanol to give a final volume of 1·5 ml. Further dilutions, as required to bring the response into the linear range of the detector, were completed with appropriate volumes of methanol + water (50 + 50 by volume).

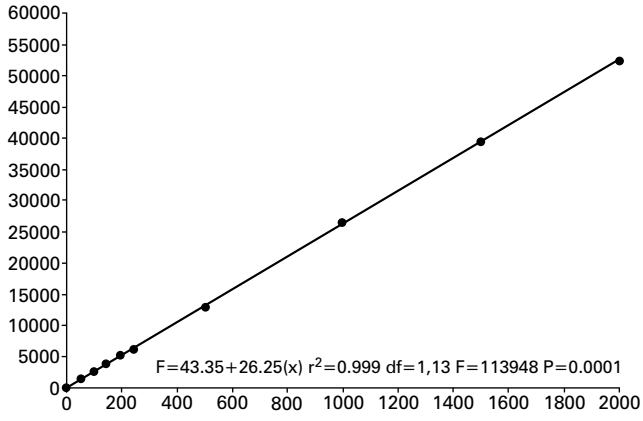

Fig. 2. Linearity of detector response to tebufenozide.

2.5 HPLC analysis

Tebufenozide deposits on current-year balsam fit foliage were quantified using reverse-phase HPLC with ultraviolet detection at $\lambda = 236$ nm. Detailed specifications for the instrumentation and operating conditions are provided in Table 2. Under these conditions, the analytical standard of tebufenozide (>99·6% Lot No. AMB9-40b; Rohm & Haas, Inc.) had a retention time of 25·45 min with 250 ng injected generating a full-scale deflection at attenuation = 8 and repeat injections ($n = 4$) exhibiting a detector response variation of <0·87% coefficient of variation. The detector response was linear (df = 1, 13; $R^2 = 0{\cdot}999$, $P = 0{\cdot}000$) over a range of 0·005 to 2 μg injected (Fig. 2). Prior to conducting field sample analyses, analytical methods were validated by fortifying blank samples with two levels of tebufenozide (1·0 and 0·5 μg g^{-1}; $n = 3$ per level). Results demonstrated good recovery efficiency and excellent precision with means ($\pm$ cv) of 86·31($\pm$ 3·64)% and 74·24($\pm$ 3·19)%, respectively.

Throughout the period of field sample analyses, a quality control (QC) program was also conducted and comprised 35 blank foliage samples fortified at several different levels ranging from 0·5 to 5 μg g^{-1} (Table 3) and equilibrated for a minimum of 18 h prior to extraction and analysis. Analysis of two blank (unfortified) samples verified the lack of co-extractive interference at the retention time of the analyte and showed no evidence of cross-contamination between samples. Linear regression analyses showed no significant trend of recovery efficiency in relation to the five different fortification levels used ($n = 35$; df = 1, 35; $P = 0{\cdot}25$). There-

TABLE 2
Instrumentation and Operating Conditions for the Analysis of Tebufenozide

Item	*Specification*			
HPLC system	Varian Model 5560 Ternary HPLC			
Detector	UV-VIS set at 236 nm			
Electronic Integrator	Varian Vista 402			
Guard column	ODS-5			
Column	Hewlett-Packard Spherisorb ODS-2 0·5 μm 250 × 4 mm			
Column temperature	40°C			
Mobile phase	Binary solvent system comprised of Solvent A = 0·005M PIC A low UV reagent[a] in 25% methanol @pH = 7·0 and Solvent B = 100% methanol			
Gradient elution method (Total run time 35 min)	Time (min)	Solvent A (%)	Solvent B (%)	Flow Rate (ml min^{-1})
	0	75	25	1·0
	18	30	70	0·8
	25	10	90	0·7
	28	0	100	1·0
	32–35	75	25	1·0
Analyte retention time	25·45 min			
Injection volume	100 μl			

[a] PICA low UV reagent = tetrabutylammonium hydrogen sulfate (Whatman Cat. No. WAT0841889, Whatman Inc. Millford, MA).

TABLE 3
Mean Recovery Efficiency and Variation for Tebufenozide-Fortified Balsam Fir Foliage Quality Control Samples

Fortification ($\mu g\ g^{-1}$)	n^a	*Mean recovery* (%)	$STDS^b$	CV^c
0·5	4	79·99	12·15	15·19
1·0	9	75·47	4·56	6·05
2·0	12	81·85	6·65	8·13
3·0	4	77·19	4·69	6·07
5·0	6	73·83	3·51	4·75
Overall	35	78·09	6·82	8·73

[a] n = number of samples.
[b] STDS = standard deviation of samples.
[c] CV = coefficient of variation (STDS/Mean × 100).

fore, the overall mean recovery efficiency (78·1%) was used to derive a factor of 1·28, which was used to correct all reported residues for analytical recovery efficiency. Limits of quantification (LOQ = 0·5 μg g^{-1}) for the analytical method were established as the lowest fortification test level meeting acceptability criterion of recovery efficiency >75% and coefficient of variation of <15%. Limits of detection (LOD = 0·1 μg g^{-1}) were conservatively established as approximately 2 × signal : noise at the analyte retention time in a blank matrix sample.

2.6 Assessing spruce budworm populations and host tree defoliation

Larval spruce budworm populations in treated and control plots were evaluated twice; once four to five days before the sprays and again 15 to 19 days after the sprays to assess population declines and calculate those that might not be natural. Because the lethal effects of tebufenozide are expressed slowly against spruce budworm, the post-spray evaluations were delayed and conducted when the budworm larvae were pupating but before any adults had emerged. The sampling procedure was as described in an earlier published report.[19] The branches were stored in a cold room (approx. 8°C) near the experimental site to inactivate the budworm larvae without causing mortality. The samples were then examined in a counting mill where the numbers of living larvae were counted. Population reductions in the treated plots were corrected for natural mortality observed in the controls.[16,20]

The sample trees in each plot were assessed visually[21] to determine the extent of defoliation caused by spruce budworm and to evaluate the treatments' effectiveness in protecting host tree foliage. The defoliation in each sample tree was ranked into ten 10-percentile classes (0–10 to 91–100%). Comparative assessments using Fettes' method[22] of random sample trees strongly corroborated the visual method.

2.7 Statistical analyses

Because the field experimental design was not truly spatially replicated, inferential statistical procedures could not be routinely used. Nevertheless, each 'block' was analysed as an independent variable and 'number of applications' and 'emitted volumes' as sources of variation. Where necessary, the data from spruce budworm population, spray deposit on Kromekote cards and foliage were transformed ($\log_{10}[x + 1{\cdot}0]$) to stabilize wide variances in the data. Defoliation and population reduction percentages were subjected to square root ($\sqrt{x + 0{\cdot}5}$) and arc sine transformations, respectively.[23] However only non-transformed means are presented in tables and figures. The data were analysed with BMDP computer software[24] using primarily analyses of variance (ANOVA). Non-parametric Mann–Whitney rank sum test was used where data failed to meet the assumption of normality ($P = 0{\cdot}05$) and multiple comparison of means, (program 7D, Bonferroni test) to separate significant differences. Regression analyses (programs 6D and 1R) were used to determine and compare relationships between variables.

Results of field AI analyses were compiled with sample means, standard deviations (SD) and coefficients of variation (CV). Sub-sample maxima and minima were calculated for each block and spray event. Selected block means were compared using a non-parametric Mann–Whitney rank sum *t*-test, as these data typically failed to meet the parametric assumption of normality ($P = 0{\cdot}05$). Correlation analysis was used to investigate the relationship between foliar deposit (μg g^{-1}) of tebufenozide on current-year balsam fir foliage and number of spruce budworm larvae on a 45-cm branch.

3 RESULTS AND DISCUSSION

3.1 Spray applications

The tank-mix concentrations ranged from 11·4% more than prescribed to 17% less than expected (Table 1). These variations are within experimental error and should be expected when quantities of a product have to be diluted with large volumes of water that might be measured imprecisely at the spray site.

The sprays were emitted at rates (litre min^{-1}) that, with one exception, exceeded what were prescribed (Table 1). However these flow rates did not relate to the emitted volume (litre ha^{-1}) as they should. The emitted volume exceeded the prescribed volume in five out of the eight applications (Table 1). Excesses, on a percentage basis occurred more in the applications that prescribed 1 litre spray ha^{-1} (24 to 38%) than in the 2-litre

spray ha^{-1} applications (5 to 23·5%). Three applications, all with prescribed volumes of 2 litre ha^{-1} spray, emitted less (1·5 to 8·5%) than expected. Nevertheless, these variations in emitted volumes and flow rates, and anomalies between the two, are considered to be within acceptable limits and were probably caused by spraying at varying air speeds. Considering the increases in emitted volumes and decreases in tank-mix concentration, the tebufenozide applications, in general, appeared to be satisfactory.

3.2 Weather

The experimental treatments were applied under favourable weather conditions (Table 4) with high relative humidity, moderate (0·75–2·0 m s^{-1}) wind-speeds and cool temperatures. The second semi-operational application treatment was conducted during a moderately warm afternoon with low (62%) relative humidity, conditions that tend to accelerate in-flight evaporation of water-based droplets.

Figure 3 shows the post-spray temperature and rainfall to which the tebufenozide deposit was exposed. Weather, in particular, rainfall and ultraviolet radiation, tends to degrade and reduce the amount of active tebufenozide on foliage.[25] Because temperatures influence spruce budworm activity, the daily minimum, mean and maximum post-spray temperatures (Fig. 3) suggest that the post-spray periods were conducive to feeding by spruce budworm larvae, and, as a consequence, their acquisition of a toxic dose of tebufenozide. Nevertheless, rainfall during the immediate post-spray period (Fig. 3) could have influenced the residual toxicity of tebufenozide.

3.3 Spray deposit on cards

All the treatments resulted in low droplet densities with wide variances between the samples (Table 5), but this might have been caused by the relatively slow speed (4200–5440 rev min^{-1}) at which the rotary atomizers were spinning. In general, approximately twice as many drops deposited on Kromekote cards from the experimental application at 2 litre spray ha^{-1} ($\bar{x} = 1{\cdot}69$ drops cm^{-2}) as from the application at 1 litre spray ha^{-1} ($\bar{x} = 0{\cdot}86$ drops cm^{-2}).

The volume and number median diameters (VMD and NMD, respectively, Table 5) suggest that, with two exceptions (the second applications of treatments E2 and S5), the spray spectra from the various strategies were moderately fine and similar. With respect to the influence of the number of applications or the litres ha^{-1} sprayed in a strategy on droplet density (measured on Kromekote cards), Fig. 4A suggests that there was only marginal interaction ($P_{vxA} = 0{\cdot}064$) between number of applications and spray volume in determining the number of drops that deposited, and that the two main effects influenced droplet density independently of each other. The sizes of the median diameters recorded from the second application of strategy S5 (Table 5) deviated greatly from those of the other strategies. This difference might have been because this spray was applied in the evening when relative humidity was sub-optimum (<65%) and the temperature was high (21°C). Such conditions favour accelerated in-flight evaporation of aqueous drops,[26] thus resulting in small drops at the depositaries.

3.4 Spray deposit on foliage

The amount of AI recovered on foliage after a pesticide spray is dependent largely on the concentration or, for example, in the case of *Bacillus thuringiensis*, the potency of the spray mix, (i.e. the AI in a drop) the number and sizes of the drops that deposit on the foliage and the stability and persistence of the deposit. In this study the tank-mixes applied as 1 litre ha^{-1} were ~1·8 times more concentrated than those sprayed at 2 litre ha^{-1} (Table 1). The VMDs, which are only broad

TABLE 4
Meteorological Conditions during Treatments with Tebufenozide

Treatment[a]	*Application*	*Wind speed (km h^{-1})*	*Temperature (°C)*	*% RH*	*Thermal air stability*[b]
E1-70 × 1 × 2	1st	7·2 (±2·0)	8·9 (±0·7)	93	N
	2nd	2·7 (±1·5)	13·4 (±0·3)	94	I
E2-70 × 2 × 2	1st	6·5 (±1·7)	7·9 (±0·4)	91	N
	2nd	2·6 (±1·6)	13·4 (±0·3)	95	I
E3-70 × 2 × 1	1st	6·5 (±1·7)	7·9 (±0·4)	94	N
E4-70 × 1 × 1	1st	6·6 (±1·7)	8·2 (±0·5)	94	N
S5-70 × 2 × 2	1st	10·6 (±2·4)	11·3 (±0·8)	90	N
	2nd	6·7 (±1·2)	21·0 (±1·0)	62	N

[a] g(AI) × litre ha^{-1} × number of application; E = experimental; S = semi-operational.
[b] N = neutral; I = inversion (see Ref. 26).

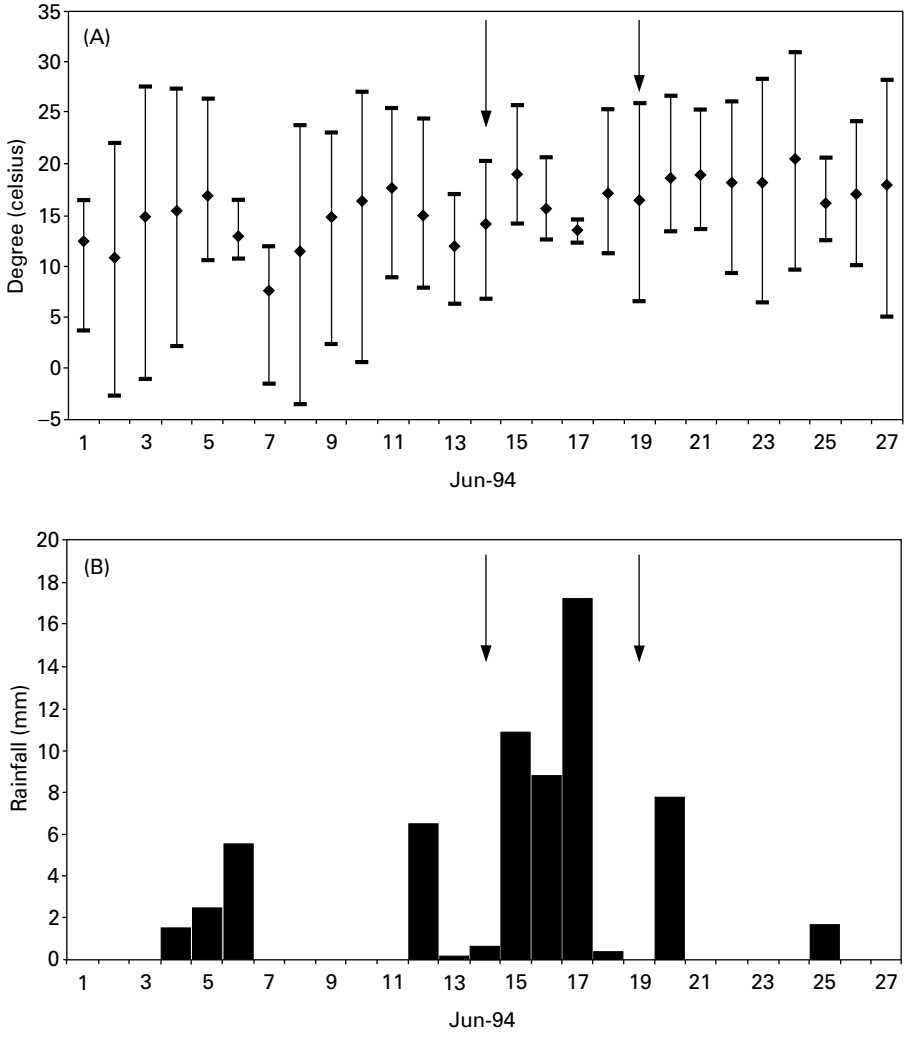

Fig. 3. (A) Temperature (mean, max, min) and (B) rainfall during the field study. ↓ denote dates of spray application.

TABLE 5
Deposit from Five Spray Treatments with Tebufenozide on *Abies balsamea* Foliage and Kromekote Cards

		Drops on Kromekote cards[b,c]							
			Diameter (μm)			*Tebufenozide on foliage (μg g^{-1})*[c]			
Treatment[a]		*No. cm^{-2} (±SD)*	*Volume median*	*Number median*	*N*	*($\bar{x}$ ± SD)*	*CV (%)*	*Max*	*Min*
E1	1st	0·29 (±0·45)a	112	72	30	3·54 (±2·73)ab	77·3	8·05	0·26
	2nd	0·46 (±0·59)ab	130	45	30	2·53 (±1·75)a	69·2	8·60	0·63
E2	1st	1·92 (±2·16)c	115	72	29	6·18 (±4·81)bcde	77·8	19·89	0·75
	2nd	0·95 (±1·15)acd	110	27	30	5·92 (±3·52)d	59·4	15·47	1·85
E3		1·20 (±1·08)ce	127	47	12	2·52 (±1·49)ae	59·1	5·17	0·40
E4		1·41 (±2·18)acf	100	64	16	5·97 (±5·57)acde	93·3	18·67	0·17
S5	1st	1·14 (±1·25)adef	102	48	20	8·53 (±8·09)cd	94·8	36·08	1·54
	2nd	0·53 (±0·37)ade	31	24	30	6·77 (±6·67)bd	99·8	35·02	0·56

[a] See Table 1 for treatment details.
[b] Where $n = 60$ Kromekote cards per treatment.
[c] Means in a column followed by unlike letters are significantly different ($P < 0·05$) using BMDP Bonferroni comparison of means analysed using transformed data (see Ref. 24). Figures shown are not transformed.

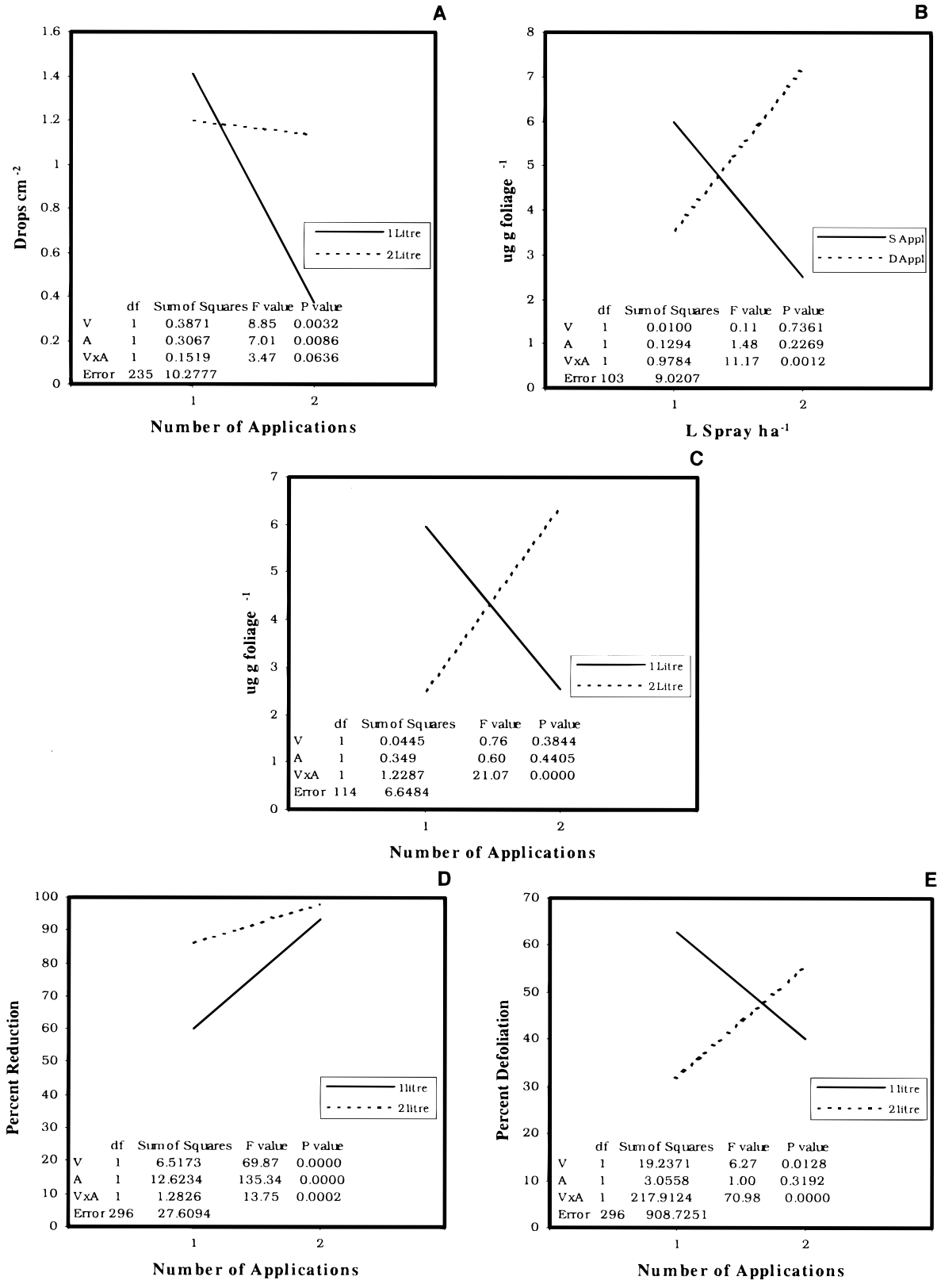

Fig. 4. Influence of interactions between number of applications and application volume on (A) droplet density, (C) cumulative foliar tebufenozide after two applications, (E) defoliation of balsam fir and (D) spruce budworm population reduction; and between (B) application volume and spray strategy on foliar tebufenozide after a single spray. ($P \leq 0{\cdot}05$ show significant interaction).

classifications of spray spectra, suggest that droplet sizes from the different treatments were not substantially different. However, droplet densities recorded on Kromekote cards were generally greater when 2 litre spray ha^{-1} were applied. Rain that fell within 24 h of applications (Fig. 3), would have caused some wash-off of tebufenozide[25] in the five-day interim between applications, which might have affected the insecticide's residual toxicity.

The mean deposit of tebufenozide for each treatment is presented in Table 5. A substantive difference in mean foliar deposit was observed between the two experimental treatments that used single applications at 70 g AI ha^{-1} (2·52 and 5·97 $\mu g\ g^{-1}$ from 2 litre and 1 litre spray ha^{-1}, respectively). Considering that the two sprays were applied within 5 min of each other (Table 1) and under essentially identical meteorological conditions (Table 4), it was expected that, if any difference did occur, the greater deposition would have favoured the treatment with 2 litre spray ha^{-1} and not vice versa. Nevertheless, when single and double application strategies were considered, as was observed with droplets on cards, more tebufenozide was recovered on new balsam fir shoots after treatments at 2 litre spray ha^{-1} than when 1 litre ha^{-1} was applied (Table 5).

When double applications of 1 or 2 litre spray ha^{-1} were used as experimental treatments (E1 & E2, respectively), foliar deposits collected after the first application were not significantly different ($P > 0{\cdot}05$) (Table 5). It is recognized that in double application treatments, residual deposit from the first sprays may persist and deposits recovered after the second application might, in fact, be accumulations of the two sprays. Nevertheless, foliar deposits recovered from the E2 (2 litre spray ha^{-1}) treatment after the second spray were significantly greater (Bonferroni test $T_{57} = 5{\cdot}82$; $P < 0{\cdot}001$) than those recovered from the E1 (1 litre spray ha^{-1}) treatment. This particular finding suggests direct relationships between application volume and foliar deposit.

However, there was significant interaction between number of applications and spray volumes that influenced the deposition of tebufenozide on foliage after the first spray (Fig. 4B) and cumulatively after two applications (Fig. 4C). Thus the effects of either of the two independent variables depend on the input level of the other and, as a consequence, suggest that simplified direct relationships between these particular variables might be tenuous.

A good relationship ($R^2 = 0{\cdot}84$; $F_{1,4} = 26{\cdot}27$; $P = 0{\cdot}004$) was observed between the droplet density and foliar tebufenozide when the treatment was a single application at 1 litre spray ha^{-1} (strategy E4, Table 6). However, weak or poor relationships were observed between flow rate (litre spray min^{-1}) and foliar AI($R^2 = 0{\cdot}30$; $F_{1,6} = 2{\cdot}52$; $P = 0{\cdot}163$) and between application volume (litre spray ha^{-1}) and foliar AI($R^2 = 0{\cdot}37$; $F_{1,6} = 3{\cdot}51$; $P = 0{\cdot}109$). Such variations in deposit probably reflect the extreme heterogeneity that characterizes both natural forests and aerial spraying and suggest that uniform spray deposit in forests might be achieved largely by chance and thus cannot easily be prescribed.

TABLE 6
Relationships between Number of Drops on Kromekote Cards and Tebufenozide Deposit on Balsam Fir Shoots

Treatment[a]	R^2	*DF*	*F*	*P value*
E1-1st	0·05	1·25	0·059	0·809
2nd	0·30	1·28	12·17	0·001
E2-1st	0·13	1·19	0·325	0·575
2nd	0·09	1·28	0·236	0·630
E3	0·07	1·10	0·044	0·837
E4	0·84	1·5	26·27	0·004
S5-1st	0·35	1·18	9·510	0·006
2nd	0·02	1·27	0·424	0·520

[a] See Table 1 for treatment details.

3.5 Spruce budworm population reduction

Percentage reductions of treated spruce budworm populations were, with one exception (i.e. populations treated with strategy E4), significantly higher ($P < 0{\cdot}05$) than reductions in the control populations (Table 7). The low coefficient of variation shown in the data from three experimental and the semi-operational blocks (Table 7) suggests that these treatments reduced the budworm populations fairly uniformly. In contrast, high coefficients of variation in data relating to treatment E4 (a single application at 1 litre spray ha^{-1}) and to the controls suggest sporadic budworm mortality among the populations.

The mean numbers of larvae that survived tebufenozide treatments were, in all cases, significantly less ($P < 0{\cdot}05$) than the mean number that survived in the controls (Table 7), indicating a high probability that the differences in survival between treated and untreated populations were not due to chance. A mean number of post-treatment spruce budworm $<2{\cdot}0$ larvae per branch suggests effective population suppression. In this study, larval budworm populations in four of five treatments were reduced to mean levels $<1{\cdot}4$ insects per branch (Table 6); however, $>7{\cdot}0$ insects per branch survived from the population that was treated with a single application at 1 litre ha^{-1} (E4). This result further corroborates the percentage population reduction results with regard to this treatment.

In general, the mean numbers of budworm larvae that survived 2 litre ha^{-1} spray volume (single and double applications) were significantly less (ANOVA $F_{2,37} = 4{\cdot}02$; $P < 0{\cdot}02$) than those that survived the 1

TABLE 7
Spruce Budworm Population Changes and Balsam Fir Defoliation following Sprays with Tebufenozide

Treatment[a]	*Spruce budworm 45-cm branch*$^{-1}$ ($\bar{x} \pm SD$)[b]		*Population reduction*[b] (%) ($\pm SD$)	*Defoliation*[b] (%) ($\pm SD$)
	Prespray	*Postspray*		
E1	26·03 (±12·2)ac	1·3 (±1·7)a	93·6 (±8·2)ab	40·0 (±22·2)ab
E2	27·6 (±16·2)ac	0·6 (±0·1)b	96·2 (±13·2)bc	48·7 (±20·62)bc
E3	14·5 (±10·6)b	1·3 (±1·4)a	85·6 (±19·1)a	31·5 (±21·2)a
E4	21·8 (±15·2)c	7·4 (±7·3)c	61·4 (±33·7)d	62·8 (±28·9)cd
S5	29·2 (±19·1)ac	0·4 (±0·8)b	98·3 (±3·3)c	62·8 (±19·2)d
Control	30·7 (±16·2)a	10·2 (±6·9)d	60·7 (±27·8)d	92·1 (±4·7)e

[a] See Table 1 for treatment details.
[b] Means in a column followed by unlike letters are significantly different ($P \leq 0{\cdot}05$) using the Bonferonni comparison of means (see Ref. 24).

litre spray ha^{-1} treatments. This result again suggests that the higher application volume might be more efficacious than a 1 litre spray ha^{-1} treatment. However, when we examined percentage population reductions, no significant differences (ANOVA $F_{2,37} = 1{\cdot}58$; $P > 0{\cdot}219$) were observed between plots treated with 1 litre and 2 litre spray ha^{-1}. In contrast, the mean numbers of larvae per branch that survived and the mean percentage population reductions observed after double application treatments (1 & 2 litre spray ha^{-1}) were significantly less (ANOVA $F_{2,37} = 4{\cdot}08$; $P < 0{\cdot}02$ and $F_{2,37} = 6{\cdot}08$; $P < 0{\cdot}005$, respectively) than those observed after treatments with single applications. These results suggest that treatments using 70 g tebufenozide in 2 litre spray ha^{-1} applied twice were more effective against spruce budworm larvae than were either a double application of 70 g AI in 1 litre spray ha^{-1} or a single application of 2 litre spray ha^{-1}. When we examined the influence of application volume and number of applications on population reduction, the data showed a lack of interaction between the two factors (Fig. 4D), which suggests that either variable could have influenced budworm population reduction independently. Because the number of drops that can be made from a unit volume of liquid varies inversely as the cube of the drop diameter,[27] e.g., one thousand 10-μm drops require the same volume as one 100-μm drop, it is therefore implicit that small (<50 μm) drops be atomized from volumes $<1{\cdot}5$ litre ha^{-1} if sufficient numbers of drops are to be available to adequately cover the foliage with AI. It is therefore likely that 1 litre spray ha^{-1} provides an insufficient number of lethal drops for tebufenozide treatments to be consistently effective against spruce budworm. The second application of a treatment strategy also offers an opportunity to correct deposit gaps that may have occurred in the first spray.

No relationship was observed between foliar tebufenozide or drops cm^{-2} on Kromekote cards and percentage budworm population reduction ($R^2 = 0{\cdot}02$ and $0{\cdot}27$, respectively), nor were there any relationships shown between deposit and larval survival ($R^2 = 0{\cdot}12$).

3.6 Defoliation

Mean defoliation of balsam fir was significantly higher ($P < 0{\cdot}05$) in the controls than in the plots treated with tebufenozide (Table 7). This suggests that we could be 95% confident that this foliage protection was due to tebufenozide. Mean defoliation $>31\%$ was observed for all treatments (Table 7), although the budworm populations were effectively suppressed. These are high levels of defoliation for areas treated with an insecticide and can probably be attributed to the deposit patterns and the insect's process of dose acquisition. Tebufenozide has minimal contact toxicity against insects; thus if it is to be an effective toxicant, it has to be ingested. If the deposit on the insect's food source, i.e. on foliage, is sparse and insufficiently toxic, the insect would need to consume more foliage to accumulate a lethal dose than if the deposit was dense and toxic. There were substantive and in some cases significant ($P < 0{\cdot}05$) differences in defoliation between the experimental treatments. The defoliation in plots treated semi-operationally was significantly different from that in three out of four experimental treatments (Table 7). High coefficients of variation observed in defoliation data are indicative of wide ranges in foliage loss between samples. It is therefore evident that the severity of defoliation was uniform in the controls, whereas the quantity of foliage eaten by budworm in the experimental treatment blocks varied widely, suggesting that in some cases the sprays might have protected some areas better than other.

In general, percentage defoliation in areas treated at 2 litre spray ha^{-1} was not significantly different (ANOVA $F_{2,37} = 1{\cdot}01$; $P > 0{\cdot}37$) from that in areas treated with 1 litre spray ha^{-1}, nor was defoliation in areas treated with a single application significantly different ($F_{2,37} = 0{\cdot}0$; $P > 0{\cdot}99$) from that in areas treated with double applications (Fig. 4E). The graphs and P value show

that significant interaction between spray volume and number of applications also affected defoliation. After experimental or semi-operational applications, no significant relationships were observed between the number of drops or between tebufenozide on foliage and defoliation ($R^2 = 0{\cdot}08$ and $0{\cdot}18$, respectively). This study has shown to what extent three primary spray variables (AI, application volume and number of applications), constituting five treatment strategies, interact to influence the deposit and efficacy of tebufenozide against spruce budworm in a natural forest.

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